Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Notification 6786-01-0183

Summary of the risk assessment of the genetically modified

potato (*Solanum tuberosum*)

within the framework of a proposed deliberate release

carried out by the German Competent Authority

Berlin, 19. April 2007

Explanatory note to this document:

The following text reflects the summary of the risk assessment of (a) genetically modified organism(s) to be used for experimental field trials (deliberate releases) in Germany. The text forms part of the official authorisation regarding applications for the permit of deliberate releases (field trials) of genetically modified organisms in Germany under the legal framework of Directive 2001/18/EC and the German Gene Technology Act (Gentechnikgesetz, GenTG). The authorisation is issued by the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL [*Federal Office of Consumer Protection and Food Safety*], as the German Competent Authority. It comprises the chapters

I. Consent [to the application]
II. Provisions [to be respected in execution of the field trials]
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III.2 Appraisal of and reply to objections
IV. Costs
V. Legal instruction

Only the original German document is legally binding. The following passage is a courtesy translation of the chapter III.1.2. and was prepared for the Biosafety Clearing House.

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III.1.2.1. Evaluation of changes in the genetically modified plants effected by the transferred nucleic acid sequence

(a) Fragments of the coding region of a potato starch synthase gene (granule-bound starch synthase, GBSS) in antisense orientation and in sense-antisense orientation (as an inverted repeat)

The fragment of the coding region of a potato starch synthase gene (granule-bound starch synthase, GBSS) in antisense orientation (plasmid pAP2) is expressed under the control of its own *gbss* promoter primarily in the potato tuber. In these genetically modified plants, the formation of an antisense RNA causes inactivation of the endogenous transcript of the respective gene, thus inhibiting production of the GBSS enzyme.

The fragment of the coding region of the potato starch synthase gene *gbss* in sense and antisense orientation (as an inverted repeat, plasmid pAP4) is also expressed under the control of its own *gbss* promoter primarily in the potato tuber. In the genetically modified plants, the formation of a double-stranded RNA is effected, which causes inactivation of the endogenous transcript of the respective gene, thus inhibiting production of the GBSS enzyme.

Due to the decreased amount of GBSS protein, a starch with reduced amylose content is synthesised in the tubers. This reduced amylose content was determined by the applicant by staining the starch granules with iodine and by spectrophotometry.

The genetically modified potatoes harvested in the field trials are not intended for use in the production of foodstuffs or animal feed. Within the scope of the proposed release the alteration of the starch composition of the genetically modified potato plants is not expected to pose any threat to human or animal health, or to the environment. No new proteins will be generated in the plant as a consequence of the genetic modification.

(b) Fragments of the coding regions of the *be1* and *be2* genes (branching enzymes) in sense and antisense orientation (as an inverted repeat)

The *be1* and *be2* genes code for branching enzymes which catalyse the splitting of α-1,4-glucans and the subsequent formation of α-1,6-glycosidic bonds between glucan chains during amylopectin synthesis.

The fragments of the coding regions of the *be1* and *be2* genes in sense and antisense orientation (as an inverted repeat) are expressed under the control of the potato´s gbss-promoter primarily in the tubers of the genetically modified plants. Transcription is terminated by the *nos* terminator from *Agrobacterium tumefaciens*.

A double-stranded RNA is formed in the genetically modified plants; RNA interference causes inactivation of the endogenous transcripts of the respective genes. As a result, formation of the corresponding enzymes is blocked and a starch with reduced amylopectin content is synthesised in the tubers.

The reduction in *be1* and *be2* expression was demonstrated by the applicant on the basis of RNA expression using real-time RT-PCR. The reduction in the amylopectin content of the starch was determined by iodine staining of the soluble starch and by spectrophotometry.

The genetically modified potatoes obtained from the proposed field trials are not intended for use in the production of foodstuffs or animal feed. The alteration of the starch composition of the genetically modified potato plants within the scope of the proposed release is not expected to pose any threat to human or animal health, or to the environment. No new proteins will be generated in the plant as a consequence of the genetic modification.

(e) Sequences located outside the T-DNA

As a general rule, only DNA located within the border regions is integrated into the plant genome in Agrobacteria-mediated transformation events. However, transfers of DNA fragments outside the border regions have been reported in the literature.

The genetically modified potato lines were obtained by transformation with the plasmids pAP2, pAP4, pHAS3, VCPMA16 or VCPMA19. These plasmids contain the following outside the border regions:

- the *aadA* gene, which confers resistance to the antibiotics streptomycin and spectinomycin,
- the *bom* site from pBR322 for mobilisation of the plasmid from *E. coli* to *Agrobacterium tumefaciens*,
- the origins of replication ColE1 and pVS1-repA for replication in *E. coli* or in *Agrobacterium*, as well as the *sta* (stability) region from pVS1.

Using a primer/probe set at both the right and left border regions, real-time PCR showed that no plasmid sequences had been integrated outside either of the border regions in the genetically modified lines intended for release. The primer/probe set at the right border region is directed at an internal sequence of the *aadA* gene. It can be assumed that the above-mentioned sequences, in particular the *aadA* gene, are not contained in the genetically modified lines.

(f) Position effects and context changes; allergenicity

The expression level of genes which have been integrated into the plant genome by genetic engineering methods depends on the site of integration on the chromosome and on the sequences neighbouring the integration site ("position effect"). Under field conditions the level of expression may be influenced by environmental factors, for instance, by temperature. In this particular case this could mean that the characteristics of the genetically modified potato plants are not modified to the same degree in the open field as under climate-controlled or greenhouse conditions. This is not expected to pose a risk to the environment or to human or animal health.

The insertion of foreign genes may influence the expression or regulation of the plant's own genes at or near the site of insertion. Such processes may alter plant metabolic pathways. However, during the course of the work carried out to date on these genetically modified plants, no observations were made that would suggest such an event.

Mobile genetic elements (transposable elements), which when transposed into the genome can exert effects on existing plant genes at the target site, occur naturally in plants. The inactivation of genes or alterations in gene regulation also take place in a range of other naturally occurring processes such as point mutations, deletions or translocations and are traditionally used in plant breeding. Therefore, even in non-genetically modified plants such events can always influence plant metabolic pathways. With regard to these properties the genetically modified plants do not differ fundamentally from non-genetically modified plants.

Given the current state of knowledge, it is not possible to make reliable predictions about the possible allergenic action of a protein on the basis of the amino acid sequence. The genetically modified potatoes are not intended for use as food or feed within the framework of the proposed release. The pollen of potato plants is only dispersed over short distances by wind and generally does not play a noteworthy role in triggering pollen allergies.

III.1.2.2. Evaluation of the ability of the genetically modified plants to persist or establish in the environment

The cultivation of potatoes in Central Europe goes back several hundred years. In areas where potatoes have been cultivated, tubers or seeds may remain in the soil after harvesting. Depending on temperatures in the winter following cultivation, these may give rise to volunteer potato plants the following year. In Europe the establishment of potatoes in natural ecosystems has not been observed, since potatoes compete poorly against wild plants and they are not frost resistant. From time to time potato plants are found beyond the cultivated areas, but only on non-natural sites such as verges and other ruderal areas. Owing to the lack of frost hardiness the cultivated potato does not establish in these areas either.

Tubers of the genetically modified trial plants will be mechanically or manually harvested, packed in sealed and marked containers, and transferred to the appropriate S1 laboratories for subsequent analysis or for storage. Surplus tuber material not intended for re-planting will be inactivated using appropriate methods, for example, steaming, autoclaving, incineration, hackling or fermentation in a biogas facility. The leaves and stalks of the potato plants will be left to decompose on the release site.

Potato plants can blossom and bear fruit. However, under Central European climate conditions there is little likelihood that potato seeds will overwinter and produce plants. Prior to harvesting, the parts of the potato plants growing above ground will be mechanically or chemically destroyed. This serves to counteract seed maturation. In the event that tubers or seeds remain in the soil, the resulting plant growth would be detected during post-trial monitoring. The crop rotation is designed in such a way that potatoes are not cultivated on the individual release sites in the following year. In overwintering experiments post-trial monitoring will be conducted from May to December of the harvest year. If genetically modified potato plants do emerge from seeds or tubers not detected during harvesting, these can be identified and inactivated by conventional agricultural practices. In such cases post-trial monitoring is extended and the release site is controlled for volunteers for a further year. No plants, or only plants which do not interfere with monitoring, may be cultivated on the release sites during the post-trial monitoring period.

In previous experiments carried out by the applicant the genetically modified potato lines did not display any significant change in appearance. Plant growth and yield did not deviate significantly from that of the control lines. One of the objectives of the proposed release is to carry out studies on the overwintering capability of the genetically modified potato tubers. For this purpose, selected parts of the experimental release crop will not be harvested in autumn, but will be left in the soil until the following spring.

Even if the genetic modification had brought about a change in the frost sensitivity of the tubers, this would be adequately addressed by the designated cultivation gap for potatoes, by post-trial monitoring and by the planned isolation measures.

There are no grounds to assume that the genetically modified potato plants have different ecological traits compared to conventionally cultivated potatoes, nor are they expected to have the ability to colonise natural ecosystems. Therefore, even if the fruit, seeds or tubers of the genetically modified plants were to be dispersed by animals, the GM potato plants would not be expected to establish in the environment.

III.1.2.3. Assessment of the possibility of pollen-mediated transfer of the inserted genes from the genetically modified plants to other plants

Attempts to cross-breed potatoes with solanaceous plants found in Central Europe were not successful. Under field conditions no incrossing took place from genetically modified potatoes to *Solanum nigrum* (black nightshade). The artificial transfer of pollen to *S. nigrum* also failed to produce viable seeds. Only under conditions that do not occur naturally and with the help of artificial methods (embryo rescue) was it possible to regenerate a small number of hybrids. These, however, turned out to be sterile. The potato and *Solanum dulcamara* (bittersweet or woody nightshade) proved to be strictly bilaterally incompatible; in crossbreeding experiments pollination of the ovule was not achieved. Similarly, the potato does not crossbreed with the tomato (*Lycopersicon esculentum*).

The following passage, therefore, deals only with a possible pollen transfer from the genetically modified potato plants to other potatoes. In agricultural practice, potatoes are propagated vegetatively via tubers. The pollen of the potato plant can be transferred by insects or by wind. However, wind dispersal only takes place over short distances.

In previous trials the genetically modified potato plants intended for release showed no significant changes in appearance when compared with conventional control lines. The minimum isolation distance of 10 m between the release sites and other agricultural areas with non-GM potatoes is considered sufficient for the purposes of the proposed trial. Should pollen be transferred to other potato plants in spite of these measures, no adverse effects are to be expected, since in an agricultural environment potato plants are propagated vegetatively, i.e. not via seeds.

As elaborated above, the probability that potentially generated seeds could give rise to plants under the given climatic conditions is very slight. In agricultural areas such plants would be eliminated in the course of conventional soil preparation practices.

III.1.2.4. Assessment of the possibility of transfer of the inserted foreign genes from the genetically modified plants to micro-organisms by horizontal gene transfer

The inserted sequences are stably integrated into the chromosomes of the recipient organisms. There is no evidence that the transfer of genetic information from plants or its expression in micro-organisms takes place under natural conditions. However, studies on the transformation ability of soil bacteria under natural conditions suggest that the transfer of plant genetic material to soil bacteria is theoretically possible, although it is assumed that a gene transfer of this type would constitute an extremely rare event.

Insofar as we assume that an exchange of genetic material between organisms which are so distantly related in terms of taxonomy is actually possible, it could be concluded that the occurrence of an exchange of heterologous genetic material does not in itself represent a safety criterion, since such an exchange could always result in the uptake of all forms of heterologous genetic material, including all forms of plant DNA.

(a) Fragments of the coding region of a potato starch synthase gene (granule-bound starch synthase, GBSS)

These gene fragments are derived from the potato, so they are commonly found in the environment. Therefore, the probability of horizontal gene transfer of these gene fragments to micro-organisms will not be any greater as a result of the field trials.

(b) Fragments of the coding region of the *be1* and *be2* genes (branching enzymes) in sense and antisense orientation (as an inverted repeat)

These gene fragments are derived from the potato, so they are commonly found in the environment. Therefore, the probability of horizontal gene transfer of these gene fragments to micro-organisms will not be any greater as a result of the field trials.

(d) The *ahas* gene

Herbicide-tolerant variants of the AHAS enzyme resulting from induced or acquired mutations are known to exist in many plant species. The AHAS enzymes of enterobacteriaceae naturally exhibit a level of sensitivity to sulfonylurea and imidazolinone herbicides which is often up to two times lower than the corresponding plant enzymes. The isoenzyme AHAS II from *E. coli*, for example, is as tolerant of sulfonylurea derivatives as the AHAS variant S653N synthesised in the genetically modified potato plants.

Therefore, any likelihood of spreading genes for herbicide-tolerant AHAS variants via transfer between bacteria or via horizontal transfer from non-genetically modified plants will not be increased as a result of the release of the genetically modified plants.

(e) Sequences located outside the T-DNA

In the genetically modified potato lines intended for release real-time PCR showed that no integration of plasmid sequences had taken place outside the right or the left T-DNA borders.

III.1.2.5. Agrobacteria used to generate the genetically modified plants

An *Agrobacterium*-mediated binary transformation system was used to generate the genetically modified plants. It was shown that the lines intended for release do not contain any backbone sequences from the vector used for transformation. It can therefore be assumed that the plants are free of all Agrobacteria used in the transformation.

In contrast to the common wild-type *A. tumefaciens*, the *Agrobacterium* strains used are “disarmed”, i.e. they no longer have the capacity to induce tumours. In the unlikely, but theoretically conceivable, event that the inserted foreign genes are transferred to a cell of another plant via these Agrobacteria, the plant would have to spontaneously regenerate into a whole, fertile plant for the foreign genes to enter the germ cells. This is the only way that these genes could be passed on to the plant offspring. Such an event is not expected to occur under natural conditions.

Assuming that the presence of small amounts of recombinant Agrobacteria in the genetically modified plants cannot be ruled out, the potential transfer by conjugation of the binary plasmids contained in the Agrobacteria to wild-type Agrobacteria (*A. tumefaciens* or *A. rhizogenes*) present in the environment would also have to be considered, since these could, in turn, pass on the foreign genes to individual cells of other plants. In the case of infection and subsequent transformation via wild-type *A. tumefaciens* or *A. rhizogenes* a crown gall or hairy root tumour would develop from the transformed plant cell. Under natural conditions such a tumour would not be expected to give rise to a plant.